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(54)【発明の名称】　核酸分子の配列決定の方法

(57)【要約】
本発明は、複数の塩基をもつ標的核酸分子の配列決定の方法を目的とする。その原則において、重合反応中における塩基の付加の一時的な順序が核酸の単分子上で測定され、即ち、配列決定される鋳型核酸分子上の核酸重合酵素の活性が実時間で追跡調査される。塩基付加の配列における各段階における核酸重合酵素の触媒活性により、どの塩基が伸長する標的核酸の相補鎖に取り込まれるかを決定することにより、配列が確定される。標的核酸分子複合体上のポリメラーゼは、標的核酸分子に沿った移動、および活性部位におけるオリゴヌクレオチドプライマーの伸長のために適切な位置において提供される。複数の標識型のヌクレオチド類似体が、標的核酸配列において異なるヌクレオチドに対し相補的な、各々識別可能な型のヌクレオチド類似体と共に、活性部位近傍に提供される。付加されるヌクレオチド類似体が活性部位にて標的核酸のヌクレオチドに相補的であるように、活性部位にて核酸鎖にヌクレオチド類似体を付加するためのポリメラーゼの使用により伸長する核酸鎖は伸長する。重合の結果オリゴヌクレオチドプライマーに付加されたヌクレオチド類似体を同定する。核酸鎖がさらに延長され、標的核酸配列を決定するために、標識したヌクレオチド類似体を提供する段階、伸長する核酸鎖を重合する段階、および付加したヌクレオチド類似体を同定する段階を繰り返す。

* NOTICES *

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1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
[Claim 1]A method of sequencing of a target-nucleic-acid molecule with two or more nucleotide bases including the following stages: A stage of providing a complex of nucleic acid polymerase and a target-nucleic-acid molecule by which orientation was carried out by being related mutually in a position suitable in order to add a complementary nucleotide analog to an active site to target nucleic acid;
A stage of providing a nucleotide analog of two or more molds near the active site which is complementary to a nucleotide from which each type of nucleotide analog differs in target-nucleic-acid arrangement;
an added nucleotide analog -- the next -- stage; which polymerizes a nucleotide analog in an active site where a nucleotide analog added is complementary to a target-nucleic-acid nucleotide so that addition of a nucleotide analog can be received
A stage of identifying a nucleotide analog added in an active site as a result of this polymerization stage; it reaches. A stage which repeats two or more these offer stages, these polymerization stages, and these identification stages of a nucleotide analog of a mold so that target-nucleic-acid arrangement may be determined.
[Claim 2]A method according to claim 1 chosen from a group which nucleic acid polymerase becomes from DNA polymerase, RNA polymerase, reverse transcriptase, and its mixture.
[Claim 3]A way according to claim 1 nucleic acid polymerase is heat-resistant polymerase.
[Claim 4]A way according to claim 1 nucleic acid polymerase is thermal inactivation nature polymerase.
[Claim 5]A method according to claim 1 chosen from RNA with a recognition site for combination of a target-nucleic-acid molecule of double stranded DNA, a single-strand DNA, a single-strand DNA hairpin, a DNA/RNA hybrid, and polymerase, and a group which consists of a RNA hairpin.
[Claim 6]A method according to claim 1 by which nucleic acid polymerase is combined with a target-nucleic-acid molecular complex in the secondary structure of a nick of a replication origin and double strand target nucleic acid or a gap, and single-stranded target nucleic acid, a binding site created with accessories protein, or single-stranded nucleic acid which a primer combined.
[Claim 7]A method according to claim 1 of providing one or accessories protein beyond it, in order to change the activity to nucleic acid polymerase.
[Claim 8]A method according to claim 7 chosen from a group which accessories protein becomes from single-stranded binding protein, primase, and helicase.
[Claim 9]A way according to claim 1 nucleic acid polymerase is pro SESHIBU (processive).
[Claim 10]A way according to claim 1 nucleic acid polymerase is un-pro SESHIBU (non-processive).

[Claim 11]A nucleotide analog Ribonucleotide, deoxyribonucleotide, A method according to claim 1 chosen from a group which consists of ornamentation ribonucleotide, ornamentation deoxyribonucleotide, a peptide nucleotide, an ornamentation peptide nucleotide, and a nucleotide

with an ornamentation phosphoric acid-sugar skeleton.
[Claim 12]A method according to claim 1 of including the following stages further: A stage which hybridizes an oligonucleotide primer in a target-nucleic-acid molecule between offer stages before an offer stage of two or more nucleotide analogs.
[Claim 13]An oligonucleotide primer Ribonucleotide, deoxyribonucleotide, A method according to claim 12 containing a nucleotide chosen from a group which consists of a nucleotide with an ornamentation ribonucleotide, ornamentation deoxyribonucleotide, peptide-nucleic-acid, ornamentation peptide-nucleic-acid, and ornamentation phosphoric acid-sugar skeleton.
[Claim 14]A method according to claim 1 of providing a nucleotide analog with a sign.
[Claim 15]A method according to claim 14 chosen from a group which a sign becomes from a chromophoric group, a fluorescence portion, an enzyme, an antigen, a heavy metal, a magnetic probe, coloring matter, a phosphorescence group, a radioactive material, a chemiluminescence portion, dispersion or a fluorescence nano particle, the Raman signal occurrence parts, and an electrochemical detection section.
[Claim 16]A way according to claim 14 a sign adheres to a nucleotide analog with the base, a sugar portion, alpha phosphoric acid, beta phosphoric acid, or gamma phosphoric acid.
[Claim 17]A way according to claim 14 a sign adheres to a nucleotide analog by a linker.
[Claim 18]A way according to claim 14 a sign adheres to a nucleotide analog without using a linker.
[Claim 19]A method according to claim 14 of including the following stages further: A stage of being between identification stages or after an identification stage, and removing a sign from a nucleotide analog before a polymerization stage in an active site of many further nucleotide analogs.
[Claim 20]A method according to claim 19 by which a removal stage is carried out by fading of a sign.
[Claim 21]A method according to claim 20 by which fading is carried out by photofading using synchrotron radiation adjusted in order to derive and adjust removal of a sign.
[Claim 22]A method according to claim 19 by which a removal stage is carried out by cutting of a sign from a nucleotide analog.
[Claim 23]A method according to claim 22 by which beta or a nucleotide analog which was carried out as for gamma sign is cut enzymatically.
[Claim 24]A way according to claim 14 each of a nucleotide analog of two or more molds has a different sign mutually identified between identification stages.
[Claim 25]A method with a sign with which nucleotide analogs of two or more molds not more than three or it differ according to claim 14.
[Claim 26]A method according to claim 14 of having a sign identified with base fluorophore, fluorophore by whom quenching was done, or the different characteristic by existence of a fluorescence nucleotide analog, although a nucleotide analog of a different mold is the same sign.
[Claim 27]A method according to claim 1 by which nucleic acid polymerase has a sign and an identification stage is carried out by detection of an interaction between this sign and a nucleotide analog.
[Claim 28]A way according to claim 27 a sign is a fluorescence resonance energy move donor or an acceptor.
[Claim 29]A method according to claim 1 by which an identification stage is carried out by an un-optical procedure.
[Claim 30]A method according to claim 1 enforced by an optical procedure in which an identification stage is chosen from a remote place micro spectrum, an approaching space micro spectrum, an evanescent wave or a waveguide exposure, nano structure enhancement, and a group that consists of those combination.
[Claim 31]A method according to claim 1 by which an identification stage is carried out by a single photon and/or multiphoton excitation, fluorescence resonance energy movement, or use of light conversion.

[Claim 32]A method according to claim 1 by which an identification stage is attained by spectrum wavelength discernment, measurement of the life time of fluorescence and separation, fluorophore identification, and/or background control.
[Claim 33]A method according to claim 32 of using a quick change between excitation mode and an irradiation source, and its combination in fluorophore identification and/or background control.
[Claim 34]A way according to claim 1 an offer stage of a complex includes the following stages: A stage which arranges either (1) oligonucleotide primer or (2) target-nucleic-acid molecules on a base material;
It is (1) in order to form a target-nucleic-acid molecular complex which a primer combined. Hybridize a target-nucleic-acid molecule to an arranged oligonucleotide primer. Or (2) a stage which hybridizes an oligonucleotide primer in an arranged target-nucleic-acid molecule; it reaches. In a position suitable for extension of an oligonucleotide primer in movement and an active site which met a target-nucleic-acid molecule, A stage of providing nucleic acid polymerase on a target-nucleic-acid molecular complex which a primer combined.
[Claim 35]A method according to claim 34 enforced when a stage of hybridization combines additionally a target-nucleic-acid molecular terminal of an opposite hand of what combined with an oligonucleotide primer with the second oligonucleotide primer arranged on a base material.
[Claim 36]Either a base material and an oligonucleotide primer or a target-nucleic-acid molecule A method according to claim 34 of combining with a corresponding ingredient of a covalent bond pair chosen from an antigen-antibody binding pair, a streptoavidin biotin bonded pair, photoactivated tie molecules, and a group which consists of a complementary nucleic acid pair, or a noncovalent bond pair reversibly or irreversibly.
[Claim 37]A method according to claim 34 which an oligonucleotide primer is arranged on a base material and a target-nucleic-acid molecule hybridizes to an arranged oligonucleotide primer.
[Claim 38]A method according to claim 34 which a target-nucleic-acid molecule is arranged on a base material, and an oligonucleotide primer hybridizes in an arranged target-nucleic-acid molecule.
[Claim 39]A way according to claim 1 an offer stage of a complex includes the following stages: Target nucleic acid is included, and a stage which arranges a double strand nucleic acid molecule which has a recognition site near the active site on a base material -- and -- A stage of providing nucleic acid polymerase in a position suitable for movement which met a target-nucleic-acid molecule on a target-nucleic-acid molecule.
[Claim 40]A way according to claim 1 an offer stage of a complex includes the following stages: A stage which arranges nucleic acid polymerase on a base material in a position suitable in order that a target-nucleic-acid complex may move relatively to nucleic acid polymerase.
[Claim 41]A base material and nucleic acid polymerase by a corresponding ingredient of a covalent bond pair chosen from an antigen-antibody binding pair, a streptoavidin biotin bonded pair, photoactivation tie molecules, and a group that consists of a complementary nucleic acid pair, or a noncovalent bond pair. A method according to claim 40 combined reversibly or irreversibly.
[Claim 42]A method according to claim 1 of arranging on a base material which can adjust nucleic acid polymerase or target nucleic acid.
[Claim 43]A method according to claim 1 of arranging nucleic acid polymerase or target nucleic acid in gel with a stoma.
[Claim 44]A method according to claim 1 of arranging target nucleic acid and nucleic acid polymerase of each other on solid support to the neighborhood.
[Claim 45]A method according to claim 1 enforced when an identification stage decreases background noise produced from an isolation nucleotide analog.
[Claim 46]a stage that an identification stage includes the following stages and of making a field corresponding to an active site pointing to the method:activation radiation according to claim 45 substantially -- and -- A stage of detecting a nucleotide analog which polymerized in an active site.

[Claim 47]A method according to claim 45 by which a nucleotide analog which polymerized in an active site by an identification stage is discriminated from an isolation nucleotide analog.
[Claim 48]A method according to claim 45 by which an identification stage is carried out in a restricted space near the active site.
[Claim 49]A method according to claim 48 by which an identification stage is carried out in nano structure.
[Claim 50]A way according to claim 49 nano structure is pan KUCHUETO (punctuate) structure and needlelike (acicular) structure which reinforce a detection stage, or resonance nano structure.
[Claim 51]A method according to claim 48 which a nucleotide analog which has not polymerized in an active site passes along a microstructure, and moves from a restricted space promptly to a restricted space.
[Claim 52]A way according to claim 51 a microstructure contains the following: Two or more channels for making it point to a different nucleotide analog to a restricted space, It reaches. A discharge channel for making material remove from a restricted space, and nano structure containing the following: A cover constituted in order to define a restricted space and to make an identification stage easy.
[Claim 53]A method according to claim 45 enforced by electromagnetic field enhancement using electromagnetic radiation reinforced [near the subject in which an identification stage has a small curvature radius near the active site].
[Claim 54]A method according to claim 45 by which an identification stage is carried out by the approaching space exposure of a cave in which a target-nucleic-acid molecule which a primer combined is located.
[Claim 55]A method according to claim 45 by which an identification stage is carried out using an optical fiber near the complex.
[Claim 56]A method according to claim 45 by which identification and reduction of a background are carried out by gate time delay (time gated delay) of photon detection.
[Claim 57]A method according to claim 1 by which a method is enforced by sequencing of a different nucleic acid molecule in a different position of plurality on an array.
[Claim 58]A method according to claim 1 enforced by stage which carries out sequencing of the same target nucleic acid continuously simultaneous, and a stage which combines output from such sequencing.
[Claim 59]A device suitable in order to carry out sequencing of the target-nucleic-acid molecule characterized by comprising the following.
A base material.
Nucleic acid polymerase or an oligonucleotide primer which is suitable nucleic acid polymerase to combine with a target-nucleic-acid molecule, or an oligonucleotide primer, and is arranged on this base material.
A microstructure which was formed including this base material and this nucleic acid polymerase, or this oligonucleotide primer in order to move promptly a marker nucleotide analog which is not located on a base material through a restricted space and which defines a restricted space.

[Claim 60]The device comprising according to claim 59:
A microstructure, Two or more channels for making it point to a nucleotide analog of a different mold to a restricted space
A discharge channel for making material remove from nano structure constituted in order to make easy identification of a nucleotide analog located on a restricted space and a base material.

[Claim 61]A device suitable in order to carry out sequencing of the target-nucleic-acid molecule characterized by comprising the following.
A base material.

Nucleic acid polymerase or an oligonucleotide primer which is suitable nucleic acid polymerase or an oligonucleotide primer in order to hybridize in a target-nucleic-acid molecule, and is arranged on this base material.
A cover constituted in order to make easy identification of a marker nucleotide analog containing this base material and this nucleic acid polymerase, or this oligonucleotide primer which defines a restricted space and is located on this base material.
An optical waveguide near the restricted space for centralizing activation radiation on a restricted space and collecting radiation from a restricted space.

[Translation done.]